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(54) Title: ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

(57) Abstract: The present invention relates to the use of effectors/regulators for Rab and Rho GTPases in *in vitro* and *in vivo* assays that recapitulate and measure the role of these effectors/regulators in membrane transport and membrane-cytoskeleton interactions in the endocytic pathway as novel targets to find therapeutic drugs to prevent or inhibit cancer cell growth and arrest cancer cell invasiveness as well as for stimulating and/or restoring endocytic transport and phagosome maturation in cells infected by intracellular parasites, which drugs are therefore useful for the therapy and optionally also the prophylaxis of cancer and infectious diseases. In addition, the present invention is also directed to kits useful as a means to detect drugs suitable as anti-cancer and anti-infectious diseases drugs.

Assay to Detect Substances Useful for the Therapy

The present invention relates to the use of effectors/regulators for Rab GTPases in *in vitro* and *in vivo* assays that recapitulate and measure the role of these effectors/regulators in membrane transport and membrane-cytoskeleton interactions in the endocytic pathway as novel targets to find therapeutic drugs to prevent or inhibit cancer cell growth and arrest cancer cell invasiveness as well as for stimulating and/or restoring endocytic transport and phagosome maturation in cells infected by intracellular parasites, which drugs are therefore useful for the therapy and optionally also the prophylaxis of 1) cancer and other proliferative (skin repair diseases such as psoriasis), invasive or cell migration disorders (endometriosis, atherosclerosis, inflammation and allergic diseases), 2) infectious (bacterial and viral) diseases, 3) diabetes, 4) Alzheimer's disease. In addition, the present invention is also directed to kits useful as a means to detect drugs suitable as anti-cancer and anti-infectious diseases drugs.

A class of molecules shown to play an important role in the regulation of intracellular transport and organelle function is represented by Rab proteins, small GTPases of the Ras superfamily, which are required in virtually every transport step which has been investigated. Similar to other GTPases, these molecules use the conformational change induced by GTP hydrolysis to regulate downstream events necessary for vesicle formation, docking and fusion. In the GTP-bound, active form Rab proteins bind to effector proteins and in this way transmit their signal to the transport machinery. For example, in membrane docking and fusion, Rab proteins regulate the activity of SNAREs. SNAREs are integral membrane proteins that by pairing on opposite membranes engaged in docking lead to membrane fusion. The pairing of SNAREs requires the activity of Rab proteins and therefore of Rab effectors. For example, endosome membrane docking requires the presence of the Rab5 effector EEA1 which, upon bridging the two opposite membranes, allows SNAREs to pair in trans, thus leading to membrane fusion (Chistoforidis et al., 1999a). The role of Rab proteins is not restricted to membrane docking and fusion but recent data provide evidence that Rab proteins also regulate the association with and motility of vesicles along cytoskeletal filaments. Membrane-cytoskeleton interactions play an important role in

bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labeled by a covalent modification or radioactivity suitable for use in the assay.

7. Use of claim 6, wherein the assay is carried out in the simultaneous presence of at least one type of GTPase and/or endosomal membrane fractions fluorescently labeled or labeled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as an anti-cancer or anti-infectious diseases drug.

8. Use of any of the preceding claims, wherein the substance useful as pharmaceutical agent is a molecule/substance that carries one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, and a quinone group; or is an organometallic compound, a sterol moiety(ies)-containing molecule, a β-hydroxy carboxylic acid, an inorganic acid or complex such as a metallocene, a nucleic acid, a cytokine, a hormone, an antibody, or an oligopeptide comprising up to 20, preferably 8, 10, or 12, amino acid residues.

9. Use of claim 8, wherein the antibody is a polyclonal or monoclonal antibody, or a fragment thereof, humanised or human, inhibitory or stimulatory, raised against and targeted towards any of the aforementioned GTPase effectors.

10. Use of claim 8, wherein the nucleic acid is genomic DNA, cDNA, or mRNA, or a fragment there, an oligonucleotide, an oligoribonucleotide, all being based on or derived from any of the GTPase effector having any of the sequences as depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, and 15, or gene therapy vectors derived from the aforementioned GTPase effector gene sequences.